UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: **March 27, 2009**

SUBJECT: Science review of resubmitted studies (MRID 47546501-03) in support of a Tolerance Exemption Petition, registration of an End-Use Product and Request for Waivers on Certain Data Requirements for Homobrassinolid and the Registration of **Homobrassinolide Technical**

Decision Number:	381556
DP Number:	357249
EPA File Symbol Number:	69361-RT
Chemical Class:	Biochemical
PC Code:	067700
CAS Number:	80483-89-2
Tolerance Exemptions:	Pending
MRID Numbers:	47546501-03

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~~THE FOLLOWING CONTAINS CONFIDENTIAL BUSINESS INFORMATION~~

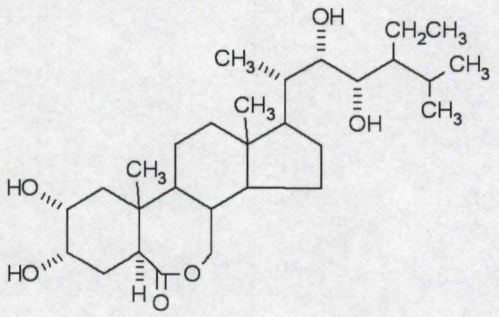
ACTION REQUESTED

1) Repar Corporation (Repar) has **resubmitted studies** in support of a tolerance exemption petition for the active ingredient, homobrassinolide(2α,3α,22S,23S,24S)-2,3,22,23-tetrahydroxy-24-ethyl-β-homo-7-oxa-5α-cholestan-6-one), on all raw agricultural commodities (food and non-food crops including forage crops and animal feed as well as residues of homobrassinolide in meat, milk, and eggs). In addition to tolerance exemptions, the registrant is also requesting waivers for all residue chemistry, and all applicable data requirements for pre- and post- harvest uses on all crops (waiver requests for all applicable guidelines Series 171).

The registrant has also submitted data in support of the registration of the product Homobrassinolide Technical. In support of the Tolerance Exemption petition and registration, the registrant has submitted product chemistry studies, Tier I toxicity studies and waiver requests, and non-target organism studies and waiver requests.

Background

Brassinosteroids are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom. These compounds, when applied to plants, improve their quality and yield and have been further researched for stress-protective properties (i.e. cold, heat, salt, and heavy metal exposure). Homobrassinolide is a plant growth regulator that is proposed as controlling or regulating the growth and development of all higher and lower plants. The proposed uses include all agronomic and horticultural crops such as agricultural crops, greenhouse food uses and non-food crops, aquatic food and non-food crops (all crops, grasses, vines, and trees listed under 40 CFR Part 158, Appendix A).



RECOMMENDATIONS AND CONCLUSIONS

1a. The following studies were resubmitted for review: Dermal Sensitization (OPPTS 870.2600); Pre-Natal Developmental (OPPTS 870.3700); and Bacterial Reverse Mutation Assay (OPPTS 870.5100).

1b. MRID 47546502: Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. **Acceptable.**

1c. MRID 475446501: Resubmission Skin Sensitization - Guinea Pigs (OPPTS 870.2600). **Acceptable**

1d. MRID 47185139: Resubmission Waiver Request for Prenatal Developmental - Rat OPPTS 870.3700). **Unacceptable**

STUDY SUMMARIES

MRID 47208904: Previous Study Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. **Unacceptable.** The doses that were tested were extremely low: the highest dose was 10^4 times lower than the limit dose of 5,000 µg/plate recommended by the Guidelines. There was no mention of toxicity caused by the test substance, with or without activation in any tester strain. However, the study is deficient and unacceptable because the researchers used a range of low doses (0.03 to 0.5 µg/plate) ignoring higher dose ranges.. In addition, only 4 of the 5 recommended strains were tested. Almost no information was provided on the S9 fraction used for activation. The number of bacteria plated for each treatment group was not presented, making it impossible to quantify the number of revertants/10^6 viable bacterial cells. No historical control data were presented.

MRID 47546502: Resubmission Bacterial Reverse Mutation Test; (Bacterial system, *Salmonella typhimurium*)/ mammalian activation gene mutation assay; OPPTS 870.5100. After range finding testing with technical homobrassinolide (87.1% ai), researchers chose five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing appeared to have been conducted according to standard protocol. Results show that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. The test substance, homobrassinolide, did not induce any apparent mutagenic effect in the *Salmonella typhimurium* strains tested. This resubmitted test is considered **Acceptable.**

MRID 47185124: Previous Study Skin Sensitization - Guinea Pigs (OPPTS 870.2600). **Unacceptable, but upgradeable** if the registrant provides a positive control study which was carried out within six months of the study and the results are appropriate.

MRID 475446501: Resubmission Skin Sensitization - Guinea Pigs (OPPTS 870.2600). None of the animals of treatment groups and control groups presented any skin irritation at 24 and 48 hour after removal of the challenge patch. This study is **Acceptable**.

MRID 47185138: Previous Waiver Request for Prenatal Developmental - Rat (OPPTS 870.3700). **Waiver denied.**

MRID 47185139: Resubmission Waiver Request for Prenatal Developmental - Rat OPPTS 870.3700). This is a Tier I requirement because this product has the potential for widespread use that can result in exposure to females. This compound is a phytosteroid with structure similar to B-sitosterol. Although the registrant provided literature information that the compound is metabolized and degraded in plant tissue, this was never quantitated and the registrant did not supply any information regarding metabolism in animals. Therefore, the prenatal developmental-rat test is a requirement for registration. The bacterial mutation assay **(MRID 47208904)** cited above is a genotoxicity study and cannot be used to fulfill OPPTS 870.3700 requirements. Therefore, the information submitted is not sufficient to support the requested waiver for teratogenicity. **Waiver denied**.

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Tier I Toxicity Studies (MRID 47208903 – 47185106, 47185118, 47185120 – 47185124, 47185127, 47208907,)

Toxicological Profile for Homobrassinolide is presented in Table 1.0 These data suggest that the compound is practically non-toxic (Toxicity Category III and IV). This compound should not cause chromosomal aberrations or cytotoxicity and is not a sensitizer or a dermal irritant. Exposure to eyes results in a mild irritation. Study summaries are listed by Guideline below (Table 1.0)

Table1.0 Toxicological Profile for Homobrassinolide Technical

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Acute Oral Toxicity – Mice (OPPTS 870.1100)	>5,000 mg/L **Acceptable**	IV	**47208903**
Acute Oral Toxicity (OPPTS 870.1100)	> 5,000 mg/kg **Acceptable**	**IV**	**47185118**
Acute Dermal Toxicity (OPPTS 870.1200)	> 2,000 mg/kg **Acceptable**	**III**	**47185120**

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
Acute Inhalation Toxicity (OPPTS 870.1300)	>2.26 mg/L **Acceptable**	**IV**	**47185121**
Acute Eye Irritation (OPPTS 870.2400)	Mild irritation **Acceptable**	**III**	**47185122**
Acute Dermal Irritation (OPPTS 870.2500)	Not an irritant **Acceptable**	**IV**	**47185123**
Skin Sensitization (OPPTS 870.2600)	No skin irritation at 48 hrs **Acceptable**	**IV**	**475446501**
90-day Oral Toxicity-rat (OPPTS 870.3100)	> 1,000 mg/L **Acceptable**	**III**	**47208906**
90-Day Dermal Toxicity (OPPTS 870.3250)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185136**
90-Day Inhalation Toxicity (OPPTS 870.3465)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185137**
Pre-natal Developmental (OPPTS 870.3700)	The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue.	**Waiver Request Denied**	**47185139**
Bacterial Reverse Mutation Assay (OPPTS 5100)	Five dosage levels ranging from 39.06 - 625 ug/plate for strains TA100, TA1535, TA98, and 9.77 - 156.25 ug/plate for strains TA102 and TA1537 with and without S9. Testing showed that there was no concentration related or reproducible increase in the number of revertant colonies in the concentrations tested and no observable statistically significant dose-response relationship. This resubmitted test is considered **Acceptable.**	No mutagenic effect in the *Salmonella typhimurium* strains tested.	**47546502**
In vivo Mammalian Cytogenetics-Erythrocyte Micronucleus assay (OPPTS 870.5395)	No a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment dose (2000 mg/kg). **Acceptable**	**Not Cytotoxic**	**47185127**

Study Type/OPPTS Guideline	$LD_{50}/LC_{50}/EC_{50}$ Results	Toxicity Category	MRID
in vitro Mammalian Cell Gene Mutation Testing (OPPTS 870.5300)	No potential for genotoxicity	**Waver Request Acceptable**	**47185132**
Mammalian Bone Marrow Chromosomal Aberration Test (OPPTS 870.5385)	No chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight. **Acceptable**	**No Chromosomal Aberrations**	**47208905**
DNA Synthesis in Mammalian Cells in Culture (OPPTS 870.5550)	Unscheduled DNA synthesis in mammalian cells in culture is not required	**Waiver is not needed**	**47185133**
in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375)	The weight of evidence from studies MRID 47208905, 47185127, and the limited information from 4708904 suggest that the information submitted is sufficient to grant a waiver for this study.	**Waver Request Acceptable**	**47185134**
Immunotoxicity (OPPTS 880.3550)	No changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function (MRID 47208906).	**Waver Request Acceptable**	**47185135**
Immune Response (OPPTS 880.3800)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185140**
Chronic Exposure (OPPTS 870.4100)	Sufficient information has been submitted to support the waiver (MRID 47208906).	**Waver Request Acceptable**	**47185141**
Carcinogenicity (OPPTS 870.4200)	A carcinogenicity test is not required	**Waiver is not needed.**	**47185142**

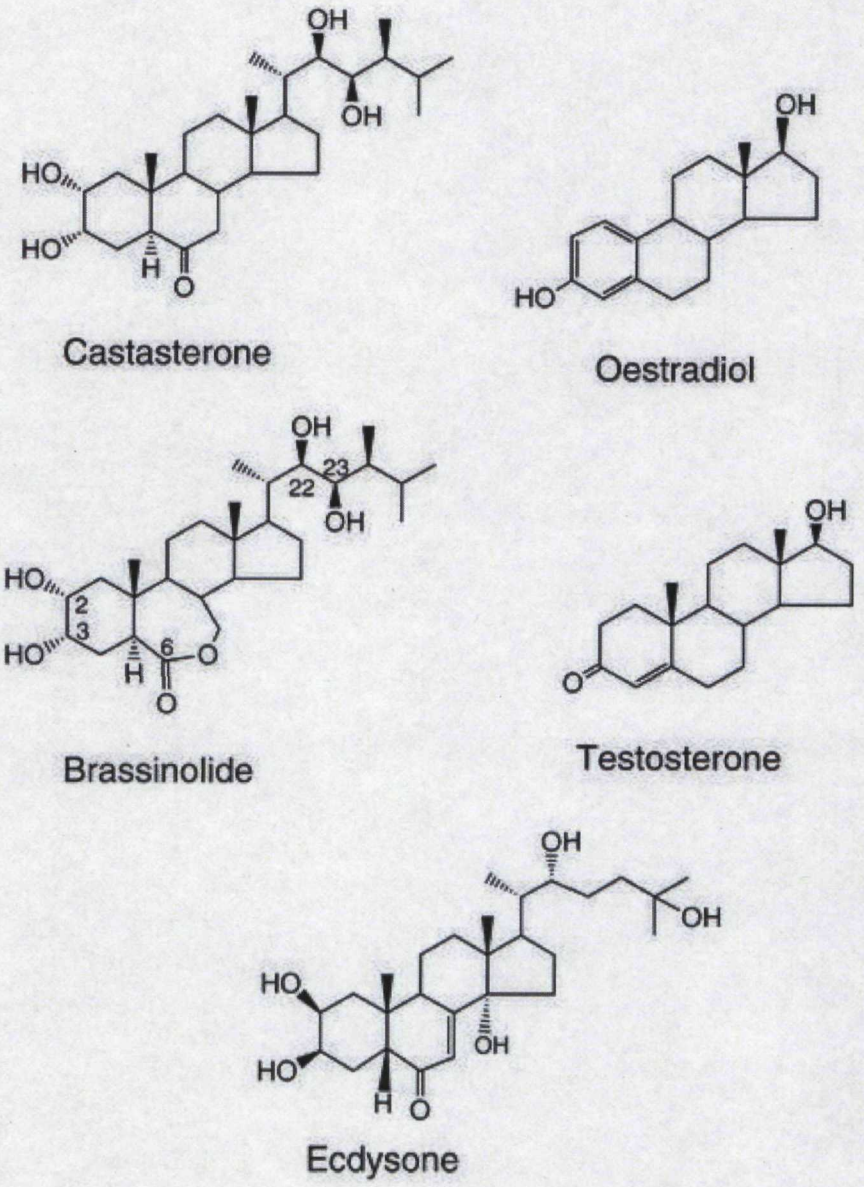

Figure 1.

Structures of Steroid Hormones.

Chemical structure of brassinolide and castasterone plant steroid hormones, in comparison with the mammalian sex steroid hormones testosterone and oestradiol, and the insect steroid hormone ecdysone. Highlighted are carbon numbers of BL having oxygen moieties that are important for BR activity.

Plant Cell. 2002; 14(Supplement): s97–s110.
doi: 10.1105/tpc.001461.